Growth and survival kinetics of *Listeria monocytogenes* in cooked egg whites

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**Abstract**

Peeled hard-boiled eggs (HBE) are ready-to-eat products susceptible to surface contamination by *Listeria monocytogenes*. This study investigated the growth and survival of *L. monocytogenes* between 4 and 43 °C in egg whites cooked under different conditions (70 °C for 15 min, 80 °C for 20 min, and 100 °C for 10 min). *L. monocytogenes* inoculated to samples cooked at 100 °C could grow uninhibitedly between 4 and 40 °C, exhibiting no lag phases, but failed to grow at 43 °C. The growth process was described by a 3-parameter logistic primary model, with the specific growth rates fitted equally well to the Ratkowsky square-root and Cardinal models. According to the Ratkowsky square-root model, the estimated minimum (nominal) and maximum growth temperatures were –0.3 and 47 °C, which were 1.6 and 44.3 °C, respectively, according to the Cardinal model. *L. monocytogenes* did not grow well when inoculated to egg white samples cooked at 70 and 80 °C. Images of scanning electron microscopy showed that *L. monocytogenes* was damaged in samples cooked at these temperatures. Although experiencing a <2 log cfu/g initial growth, *L. monocytogenes* was inhibited in these samples at all storage temperatures, probably due to the antimicrobial activities of heat-denatured and polymerized lysozyme formed at 70 and 80 °C, which were absent in samples cooked at 100 °C.

The results of this study showed that cooking temperature affected the survival and growth of *L. monocytogenes* in cooked egg whites, suggesting that HBEs may be cooked at a lower temperature in order to retain the antilisterial activities. The mathematical models developed in this study can be used to predict the growth and survival of *L. monocytogenes* in HBEs and for conducting risk analysis of this type of products.

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**1. Introduction**

According to the statistics from the USDA Economic Research Service (USDA ERS, 2012), the United States is the second-largest egg producer in the world with over 90 billion eggs produced annually. Over 75% of the U.S. egg production is for human consumption with a per capita consumption of around 250 eggs. Many types of egg products are produced, including raw or pasteurized whole eggs, whites, yolks, and various blends with or without non-egg ingredients (Mukhopadhyay, Tomasula, Luchansky, Porto-Fett, & Call, 2010). Hard-boiled eggs (HBEs) are fully cooked, usually peeled ready-to-eat (RTE) products, sold as whole eggs in pouches or trays, or used as an ingredient in other value-added products, such as salads and sandwiches.

As with all egg-containing products, *Salmonella* is a major pathogen of concern and must be destroyed prior to consumption. According to the recommendation from the U.S. Food and Drug Administration (U.S. FDA), raw shell eggs broken for immediate preparation and service must be cooked to 63 °C for 15 s and foods prepared with raw shell eggs not broken for immediate preparation and service must be cooked to 68 °C for 15 s to prevent egg-borne *Salmonellosis* (U.S. FDA, 2002). According to the advice from the USDA Food Safety and Inspection Service (USDA FSIS), casseroles and other dishes containing eggs should be cooked to a safe minimum internal temperature of 71.1 °C (USDA FSIS, 2011). Based on these recommendations, HBE products should be cooked to an internal temperature of 68 °C for at least 15 s or 71.1 °C to ensure microbial safety. According to Humphery, Greenwood, Gilbert, Rowe, and Chapman (1989), boiling eggs for 10 min could achieve >7 log-reductions of *Salmonella* Enteritidis PT4. After cooking, HBEs should be...
free of foodborne pathogens and are ready-to-serve and RTE. In the industry, the boiling process can take as much as 18 min in 97.5 °C hot water to achieve uniform heating in a commercial egg cooker (Sanovo Technology Group, 2013).

As with all RTE foods, *Listeria monocytogenes* is a major foodborne pathogen of concern. Thermally processed HBEs, free of *Salmonella* spp. immediately after cooking, can be re-contaminated with *L. monocytogenes* in the post-processing environments. A study reported by Claire et al. (2004) showed that *L. monocytogenes* can grow in HBEs packaged under various gas atmospheres at both refrigerated (4 °C) and temperature abuse storage conditions (8 and 12 °C). Recently, contamination occurred and a major U.S.-based egg manufacturer recalled HBEs from the market due to discovery of *L. monocytogenes* (U.S. FDA, 2012a). Products made from contaminated HBEs were also recalled (U.S. FDA, 2012a, 2012b) across 34 states in the United States. More than a million HBEs were recalled as a result. While most research of HBEs focuses on the safety of products concerning *Salmonella*, the objectives of this research were directed to investigate the growth kinetics of *L. monocytogenes* in HBEs, in view of the recent incidence of contamination of *L. monocytogenes* in these products (U.S. FDA, 2012a). Since the contamination of *L. monocytogenes* in HBEs primarily occurs on egg white surfaces, this study was conducted specifically in cooked egg whites at different storage temperatures and attempted to describe its growth behaviors via predictive mathematical modeling. The mathematical models developed from this study may serve as a scientific basis and assist the egg industry to produce safer HBE products and for regulatory agencies to conduct risk assessments of HBEs exposed to various temperature-abuse conditions.

2. Materials and methods

2.1. Preparation of bacteria

Five rifampicin-resistant *L. monocytogenes* strains, including three strains of *L. monocytogenes* 4b (F2365, H7858, and ATCC 19115), one strain of *L. monocytogenes* 1/2b (F4260), and one strain of *L. monocytogenes* 1/2a (V7), were obtained from the stock culture collection of the Eastern Regional Research Center (ERRC) of the USDA Agricultural Research Service (ARS) located in Wyndmoor, PA (Fang, Liu, & Huang, 2013). Each strain of *L. monocytogenes* was resistant to 100 mg/L of rifampicin (Sigma, R 3501-5G, Sigma—Aldrich Co., MO) in Brain Heart Infusion broth (BHI broth, BD/Difco Laboratories, Sparks, MD). Fresh cultures were prepared for each experiment. Stock cultures were prepared by streaking each strain of the overnight culture onto Tryptic Soy agar (TSA/BD/Difco) plates containing 100 mg/L rifampicin (TSA/R). The stock cultures were kept at 4 °C in a refrigerator and transferred every 2–3 weeks to maintain viability.

One day prior to an experiment, each strain (a 1-μl loop) was inoculated to 10 ml of BHI broth supplemented with 100 mg/L rifampicin and held at 37 °C in an orbital shaker (~100 rpm) for approximately 22–24 h. Each overnight culture was harvested by centrifugation (2400 × g, 15 min, 4 °C), washed once with 10 ml of 0.1% peptone water (PW, BD/Difco), and re-suspended in 5 ml PW. The five individual strain bacterial cultures were combined to form a cocktail, which contained approximately 10<sup>9</sup>–10<sup>8</sup> cfu/ml of *L. monocytogenes*.

The results of a preliminary study showed that there was no difference in the growth behaviors of natural and rifampicin-resistant strains of *L. monocytogenes* in cooked egg whites and therefore, the antibiotic resistant strains of *L. monocytogenes* were used to inoculate cooked egg white samples.

2.2. Egg whites and sample preparation

For HBEs, the contamination of *L. monocytogenes* occurs primarily on the surfaces of the coagulated egg white layer, which may prevent *L. monocytogenes* from entering egg yolks. Therefore, this investigation was conducted using egg whites as test samples. Pasteurized 100% all natural liquid egg whites in 32 oz cartons (907 g) purchased from a local grocery store were used in the experiments. Although the egg white was previously pasteurized and free of foodborne pathogens when purchased, all samples were tested for potential presence of *L. monocytogenes* by direct plating (0.1 ml) onto PALCAM *Listeria* agar (PALCAM agar, BD/Difco) plates (37 °C, 48 h). The opened egg white cartons were refrigerated at 4 °C and used within two weeks of purchase. All samples were tested negative for *L. monocytogenes* prior to being used in the experiments.

Pasteurized liquid egg whites (5 ± 0.1 g) were aseptically weighed into sterile sampling bags (Whirl-Pak<sup>™</sup>, 207 ml, 95 mm × 180 mm × 0.08 mm, NASCO — Fort Atkinson, Fort Atkinson, WI). The water vapor and oxygen transmission rates of the filter bags were 7.8 g/m<sup>2</sup>/day and 3100 ml/m<sup>2</sup>/day, respectively, according to the manufacturer. Thirty-six (36) egg white samples were prepared and divided into three groups, each containing 12 samples.

To evaluate the severity of heat treatment during cooking of shell eggs on the survival of *L. monocytogenes* in cooked eggs, egg white samples were coagulated at different temperatures and times in a hot water bath. The first group of egg white samples was heat-treated at 100 °C for 10 min (Humphery et al., 1989). The second group was heat-treated at 80 °C for 20 min. The third group received a treatment at 70 °C for 15 min. The first group received the most severe cooking and the third group the least.

After heat treatment, each sample was spread-inoculated with a 0.1-ml aliquot of appropriately diluted *L. monocytogenes* cocktail. After inoculation, all bags were labeled and hand-shaken for 1 min to disperse the bacterial cells. The initial concentration of *L. monocytogenes* in cooked egg whites was ca. 10<sup>7–3</sup> cfu/g, determined by direct plating onto PALCAM agar plates.

2.3. Growth study

The inoculated cooked egg white samples were placed in incubators maintained at 4, 8, 12, 16, 20, 25, 30, 33, 37, 40, or 43 °C. Samples were removed from the incubators at pre-determined time intervals according to incubation temperatures. On average, 10–12 sampling points were used for each growth curve at each incubation temperature. For enumeration of bacteria after incubation, 10 ml PW was added to each sample bag. The samples were stomached for 2 min each side at the maximum speed in a stomacher (Model BagMixer<sup>™</sup> 100W, Interscience Co., France). A small volume (0.1 or 1 ml) of the liquid portion of the stomached samples was plated, either directly or after serial dilution, onto freshly prepared TSA/R. The TSA plates were used to enumerate total aerobic bacterial counts (TAB) and TSA/R plates for *L. monocytogenes* in the cooked egg white. Both TSA/R and TSA plates were held in an incubator maintained at 37 °C for 22–24 h. The bacterial colonies were counted and converted to the logarithm of base 10 and recorded log cfu/g. Two replicates of growth experiments were conducted for each temperature condition. As a quality control procedure, the control and inoculated samples were plated periodically onto PALCAM *Listeria* agar plates (BD/Difco) to check for recovery of *Listeria* cells.

2.4. Growth models

For egg white samples heat-treated at 100 °C, the inoculated *L. monocytogenes* began to grow immediately without experiencing
lag phases. The bacterial growth curves exhibited two distinctive phases — the exponential and stationary phases. For these growth curves, a three-parameter logistic equation (Fang, Gurtler, & Huang, 2012) was used to model the growth of *L. monocytogenes* in cooked egg whites. This model is expressed as:

\[
y(t) = y_0 + y_{\text{max}} - \ln \left( \exp(y_0) + \exp(y_{\text{max}}) \right) - \exp(y_0) \exp(-\mu_{\text{max}}t)
\]

(1)

In equation (1), \(y(t)\) is the natural logarithm (Ln) of bacterial counts (N) at time \(t\); \(y_0\) and \(y_{\text{max}}\) are the natural logarithms of the initial and the stationary phase bacterial counts, respectively; \(\mu_{\text{max}}\) is the specific growth rate (h\(^{-1}\)); equation (1) is particularly suitable for growth curves without lag phases.

2.5. Effect of temperature on bacterial growth

Two mathematical equations were used to describe the effect of storage temperature on the growth rate of *L. monocytogenes* in egg white samples treated at 100 °C. The first equation was a Ratkowsky square-root model (equation (2), Ratkowsky, Lowry, McMeekin, Stokes, & Chandler, 1983). In equation (2), \(T\) is the incubation temperature (°C), \(a\) and \(b\) are coefficients; \(T_0\) and \(T_{\text{max}}\) are the estimated nominal minimum and maximum growth temperatures (°C).

\[
\sqrt{\mu_{\text{max}}} = a(T - T_0)\{1 - \exp(b(T - T_{\text{max}}))\}
\]

(2)

The second model was a Cardinal model (equation (3), Rosso, Lobry, & Flandrois, 1993). This model contains \(\mu_{\text{opt}}\), which is the estimated optimum specific growth rate observed at the optimum temperature, \(T_{\text{opt}}\), \(T_{\text{min}}\) and \(T_{\text{max}}\) are the estimated minimum and maximum growth temperatures.

\[
\mu_{\text{max}} = \frac{\mu_{\text{opt}}(T - T_{\text{max}})(T - T_{\text{min}})^2}{(T_{\text{opt}} - T_{\text{min}})(T_{\text{opt}} - T_{\text{max}})(T_{\text{opt}} - T_{\text{min}} - 2T)}
\]

(3)

2.6. Data analysis and modeling

The growth curves were analyzed using an open-source statistical analysis package R (Version 2.13.2, www.r-project.org). All nonlinear regression and data analysis (equations (1)—(3)) were accomplished using the “port algorithm” in the nls (Nonlinear Least Squares) package of R.

2.7. Scanning electron microscopy (SEM)

At the end of the storage studies (37 °C), the bacterial samples were examined using scanning electron microscopy. Fifty (50) µL aliquots of bacterial suspensions recovered from egg white samples were deposited on glass coverslips and, after one hour, the coverslips were immersed into a multi-well plate containing 5 ml of a fixative solution (2.5% glutaraldehyde–0.1 M imidazole buffer solution, pH 7.2). The multi-well plate was covered and sealed for at least 2 h before the samples were further processed.

Subsequently, the fixative solution was exchanged with 0.1 M imidazole buffer. The samples were dehydrated by successive immersion in 5 ml of ethanol solutions (50%, 80%, and absolute ethanol), with three changes at each concentration. Finally, the coverslips were critical point dried from liquid CO2 in a DCP-1 Critical Point Dryer (Denton Vacuum, Inc., Cherry Hill, NJ). After mounting on specimen stubs with carbon adhesive tabs, the coverslips were sputter coated with a thin layer of gold using a Scan-Coat Six Sputter Coater (BOC Edwards, Wilmington, MA).

Digital images of the topographical features of the bacteria samples were collected using a Quanta 200 FEI environmental scanning electron microscope (FEI Co., Inc., Hillsboro, OR) operated in the high vacuum/secondary electron imaging mode at an accelerating voltage of 10.0 kV and instrumental magnification 15,000× and 25,000×.

3. Results and discussion

3.1. Growth of *L. monocytogenes* in cooked egg white

After treatment at 70, 80, and 100 °C for the predetermined duration, liquid egg white samples were heat-coagulated and pasteurized. No bacterial cells, including *L. monocytogenes*, were recovered from uninoculated cooked samples using the direct plating method. For inoculated samples, no background microorganisms were recovered from TSA plates during the entire incubation under all storage temperature conditions. Only *L. monocytogenes* cells were recovered on TSA/R and PALCAM agar plates.

For egg white samples treated at 100 °C, the population of the inoculated *L. monocytogenes* cells started to increase immediately at all storage temperatures between 4 and 40 °C (Figs. 1—3). When the inoculated samples were incubated at 43 °C, however, the population of *L. monocytogenes* started to decline steadily after experiencing a < 1 log cfu/g initial increase (Fig. 4).

As clearly demonstrated in Figs. 1—3, the growth of *L. monocytogenes* in egg white samples treated at 100 °C did not exhibit noticeable lag phases when stored at temperatures between 4 and 40 °C, and the population of *L. monocytogenes* began to increase exponentially until the stationary phases were established. Depending on storage temperatures, the maximum cell density ranged from 5 to 8 log cfu/g in the egg white samples previously cooked at 100 °C.

Under all storage temperatures between 4 and 43 °C, the growth of *L. monocytogenes* inoculated to egg white samples cooked at 70 and 80 °C was poor (Figs. 1—4), unlike growth in egg white samples treated at 100 °C. At each temperature, a small increase in the population of *L. monocytogenes* was initially observed in the samples; however, no full growth curves were observed. The growth of *L. monocytogenes* was halted and at some temperature conditions, the population of *L. monocytogenes* actually declined.

3.2. Scanning electron microscopy and potential effect of lysozyme

Since all egg white samples were all heat-coagulated, it was hard to explain the difference in the growth behaviors of *L. monocytogenes* among the samples cooked at different temperatures. Therefore, SEM was used to observe the difference in the cells of *L. monocytogenes* inoculated to cooked egg white samples. Fig. 5 illustrates the images of *L. monocytogenes* found in the samples of cooked egg whites at the end of storage at 37 °C. Both
Fig. 1. The effect of storage temperature (4, 8, 12, and 16 °C) on the growth and survival of *L. monocytogenes* in heat-treated egg white samples (Legends: ◦, △, and ○ — raw growth data obtained from samples cooked at 70, 80, and 100 °C, respectively; solid continuous curve — equation (1) (100 °C); broken continuous curves — equation (6) (70 and 80 °C)).

Fig. 2. The effect of storage temperature (20, 25, 30, and 33 °C) on the growth and survival of *L. monocytogenes* in heat-treated egg white samples (Legends: ◦, △, and ○ — raw growth data obtained from samples cooked at 70, 80, and 100 °C, respectively; solid continuous curve — equation (1) (100 °C); broken continuous curves — equation (6) (70 and 80 °C)).
Fig. 3. The effect of storage temperature (37 and 40 °C) on the growth and survival of *L. monocytogenes* in heat-treated egg white samples (Legends: ◆, ▲, and ○ — raw growth data obtained from samples cooked at 70, 80, and 100 °C, respectively; solid continuous curve — equation (1) (100 °C); broken continuous curves — equation (6) (70 and 80 °C)).

Fig. 5A) and B) show that non-intact, significantly damaged cells of *L. monocytogenes* were observed in the samples heated at 70 and 80 °C at the end of storage at 37 °C, while typical healthy, intact, rod-shaped cells of *L. monocytogenes* were observed in the samples treated at 100 °C (Fig. 5C).

Fig. 4. The survival of *L. monocytogenes* at 43 °C in heat-treated egg white samples.

Fig. 5. SEM images of *L. monocytogenes* inoculated to cooked egg white samples. A) A *L. monocytogenes* cell found in a sample previously treated at 70 °C. B) A *L. monocytogenes* cell found in a sample previously treated at 80 °C. C) Two *L. monocytogenes* cells found in a sample previously treated at 100 °C.
In egg whites, lysozyme occurs naturally and is effective in inhibiting Gram-positive bacteria, such as *L. monocytogenes* (Hughes & Johnson, 1987; Hughes, Wilge, & Johnson, 1989). Lysozyme is a relatively heat-stable enzyme (Nohara, Mizutani, & Sakai, 1999) and may not be easily destroyed if not exposed to severe heating. According to Makki and Durance (1996), lysozyme exhibited remarkably high thermal stability at 75°C and 82°C, but not at 91°C. At pH 9.00, the D value for lysozyme was about 14 min at 75°C and 80°C, but only 4.3 min at 91°C. During thermal treatment, lysozyme starts reversible unfolding at 60°C, and is completely unfolded at temperatures above 80°C (Nohara et al., 1999). Lysozyme is found in nature as a monomer, or as a single-chain polypeptide of 129 amino acids, but it can become more active in a dimeric or polymeric form (Cegielska-Radziejewska, Lesniewski, & Kijowski, 2008). Ibrahim, Higashigushi, Juneja, Kim, and Yamamoto (1996) reported that, while irreversibly denatured at 80°C at pH 7.2, lysozyme gained increased antimicrobial activities against Gram-negative bacteria and retained its inherent activity against Gram-positive bacteria. This study suggested that lysozyme’s antibacterial activity is independent of its enzymatic activity and the denatured lysozyme, in its dimeric form, has an intrinsic structural motif that causes bacterial death through membrane perturbation (Ibrahim et al., 1996). Later, Ibrahim, Matsuzaki, and Aoki (2001) further found genetic evidence and demonstrated that lysozyme’s antibacterial activity is attributable to its structural factors. Increased antilisterial activities of lysozyme in BHI broth at elevated temperatures (70 and 100°C, 5 min) were also reported by Iucci, Patrignani, Vallicelli, Guerzoni, and Lanciotti (2007). It was possible in this study that the lysozyme’s antilisterial activities were not inactivated during heating at 70 and 80°C, but destroyed after treatment at 100°C for 10 min in this study. Since lysozyme is abundant in egg whites, it was highly possible that the suppressed growth of *L. monocytogenes* in egg white samples cooked at 70 and 80°C was caused by the antibacterial activity of heat-treated lysozyme. However, lysozyme did not exert its antilisterial action immediately after incubation was started. Instead, there was a delay in the antilisterial action of lysozyme, which allowed the population of *L. monocytogenes* inoculated to cooked egg white samples to increase slightly (Figs. 1–3). Since the antilisterial activity of lysozyme was most likely significantly destroyed by heating at 100°C, *L. monocytogenes* cells grew well white samples previously cooked at 100°C (Figs. 1–3).

### Table 1

| Model       | Parameter | Estimate | Std. Error | t value | Pr(|t|) |
|-------------|-----------|----------|------------|---------|--------|
| Ratkowsky   | a         | 2.45 × 10^{-2} | 1.63 × 10^{-3} | 15.3   | 7.39 × 10^{-11} |
|             | b         | 1.52 × 10^{-1} | 4.51 × 10^{-2} | 3.38   | 3.85 × 10^{-3}  |
|             | T_0 (°C)  | -0.30     | 0.83       | -0.36  | 0.72   |
|             | T_{max} (°C) | 47.0     | 1.73       | 27.2   | 8.14 × 10^{-15} |
| Cardinal    | T_{min} (°C) | 1.62     | 2.23       | 0.73   | 0.48   |
|             | T_{max} (°C) | 44.3     | 1.77       | 25.1   | 2.90 × 10^{-14} |
|             | T_{opt} (°C) | 34.9     | 0.62       | 55.9   | <2 × 10^{-16}   |
|             | μ_{opt}   | 5.24 × 10^{-1} | 1.81 × 10^{-2} | 29.0   | 2.92 × 10^{-15} |

3.3. Mathematical modeling of bacterial growth

3.3.1. Growth of *L. monocytogenes* in egg white samples previously cooked at 100°C

All growth curves of *L. monocytogenes* in egg white samples cooked at 100°C at temperatures between 4 and 40°C can be described using the three-parameter growth model (equation (1)), as shown in Figs. 1–3. Based on the growth rates determined using the data obtained from the incubations studies between 4 and 40°C, the effect of temperature on the growth of *L. monocytogenes* in egg white samples can be described by both the Ratkowsky square-root and the Cardinal models (Fig. 6). Table 1 lists the coefficients of these two models. According to the Ratkowsky model, the minimum (nominal) and maximum growth temperature of *L. monocytogenes* in egg white samples previously cooked at 100°C were −0.30 and 47.0°C, respectively. The minimum, optimum, and maximum temperatures estimated by the Cardinal model were 1.6, 34.9, and 44.3°C, respectively. According to ICMSF (1996, 148 pp.), the minimum and maximum growth temperatures for *L. monocytogenes* are −0.4 and 45°C, respectively. Both secondary models are equally suitable for evaluating the effect of temperature on the growth of *L. monocytogenes* in egg whites coagulated at 100°C.

It appears that storage temperature affects the maximum cell density (log_{10}(N_{max})) attainable in egg white samples cooked at 100°C (Fig. 7). The log_{10}(N_{max}) was relatively stable at storage temperatures between 10 and 40°C, but decreased significantly storage temperatures below 10°C or above 40°C, as shown in Fig. 7. This observation can be described by an empirical equation, expressed in equation (4). Table 2 lists the coefficients for equation (4).

\[
\log_{10}(N_{max}) = ae^{-\gamma(T-\beta)^4}
\]  

(4)

3.3.2. Growth of *L. monocytogenes* in egg white samples previously cooked at 70 and 80°C

The growth of *L. monocytogenes* in egg white samples previously cooked at 70 and 80°C can be divided into two stages. In the first stage, a small increase in the population of *L. monocytogenes* was observed, after which the growth of *L. monocytogenes* was inhibited in the second stage. For these growth curves, the growth and survival of *L. monocytogenes* can be described by the following differential equation:

\[
\frac{dN}{dt} = k_1 N_f - k_2 N (1-f), \quad f = \frac{1}{1 + e^{(t-t_c)}}
\]  

(5)

In equation (5), N is the bacterial count (cfu/g), and the natural algorithm of which is y(t). The parameters k_1 and k_2 are the growth rate in the first stage, and the survival rate in the second stage. The...
coefficient \( f \) is a transition function that ranges from 1 to 0, and \( t_2 \) is the critical time at which the first stage transited into the second stage. Integrating equation (4) between \( t = 0 \) and \( t = t \) yields

\[
y(t) = y_0 + k_1 t - (k_1 + k_2) \times \ln \frac{\exp(t_2) + \exp(t)}{1 + \exp(t_2)} \quad (6)
\]

This equation satisfactorily described the growth and survival of \( L. \) monocytogenes in egg white samples cooked at 70 and 80 °C (Figs. 1–3). Statistically, the growth rates of \( L. \) monocytogenes observed during the first stage of growth in egg white samples previously treated at 70 and 80 °C were not significantly different \((p = 0.438)\) from those observed in the exponential phase of growth in the samples previously treated at 100 °C. Overall, the relative growth of \( L. \) monocytogenes during the first stage of growth in samples treated at 70 and 80 °C was generally \(<2 \log \text{cfu/g}\). In the second stage, the growth of \( L. \) monocytogenes was inhibited, and the bacteria started to die off at the rate of \( k_2 \). Fig. 8 shows the effect of storage temperature on \( k_2 \).

4. Conclusions

This study was conducted to investigate the growth of \( L. \) monocytogenes in heat-coagulated egg whites. The growth experiments showed that \( L. \) monocytogenes inoculated to samples previously treated at 100 °C for 10 min experienced uninhibited growth at temperatures between 4 and 40 °C, but died off after limited initial growth \((<1 \log \text{cfu/g})\) at 43 °C. The growth of \( L. \) monocytogenes in these samples exhibited no lag phase at any growth temperature and began to grow exponentially until the stationary phase was attained. This process can be accurately described by a 3-parameter primary growth model (equation (1)). The growth rates derived from the primary model can be fitted equally well to the Ratkowsky square-root model and the Cardinal model. According to the Ratkowsky square-root model, the estimated minimum (nominal) and maximum growth temperatures were –0.3 and 47 °C. By the Cardinal model, the estimated minimum, optimum, and maximum temperatures were 16, 34.9, and 44.3 °C, respectively. Either model, the Ratkowsky square-root model or the Cardinal model, can be used to describe the temperature-dependence of the growth of \( L. \) monocytogenes in egg whites previously cooked at 100 °C. These models may be used to evaluate the effect of temperature on the growth of \( L. \) monocytogenes during risk assessments of this pathogen in HBEs during storage and distributions.

At temperatures between 4 and 43 °C, the growth of \( L. \) monocytogenes inoculated to egg white samples previously cooked at 70 °C (15 min) and 80 °C (20 min) was apparently inhibited, although limited growth \((<2 \log \text{cfu/g})\) was observed immediately after inoculation. The inhibition of \( L. \) monocytogenes was most likely attributed to the antilisterial activities of heat-treated lysozyme in egg white samples previously cooked at 70 and 80 °C. SEM images showed that \( L. \) monocytogenes cells were apparently damaged in samples heat-treated at 70 and 80 °C, while normal, healthy cells were observed in samples treated at 100 °C.

The observations of this study may benefit the egg industry that produces peeled HBEs for RTE applications, which are at risk of contamination by \( L. \) monocytogenes. Since commercial production of HBEs usually occurs at temperature close to the boiling point of water (\(>97 \degree C\)) for up to 18 min (Sanovo Technology Group, 2013), the severity of the cooking conditions may destroy the antilisterial activities of lysozyme. Thus, reduced heating during preparation of HBEs may improve the bacterial safety of the final products. More studies are underway to evaluate the effect of cooking conditions on the survival of \( L. \) monocytogenes in HBEs using actual shell eggs.

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